

BIOMARKERS

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Exposure Studies Meeting

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What is a Biomarker?

- Chemicals, metabolites of chemicals, enzymes, and other biochemical substances found in tissues and body fluids
- Measurements provide data linking **exposure** with internal dose and outcome; relevant to process of risk assessment

Biomarkers and Risk Assessment: Concepts and Principles, World Health Organization, Geneva, 1993

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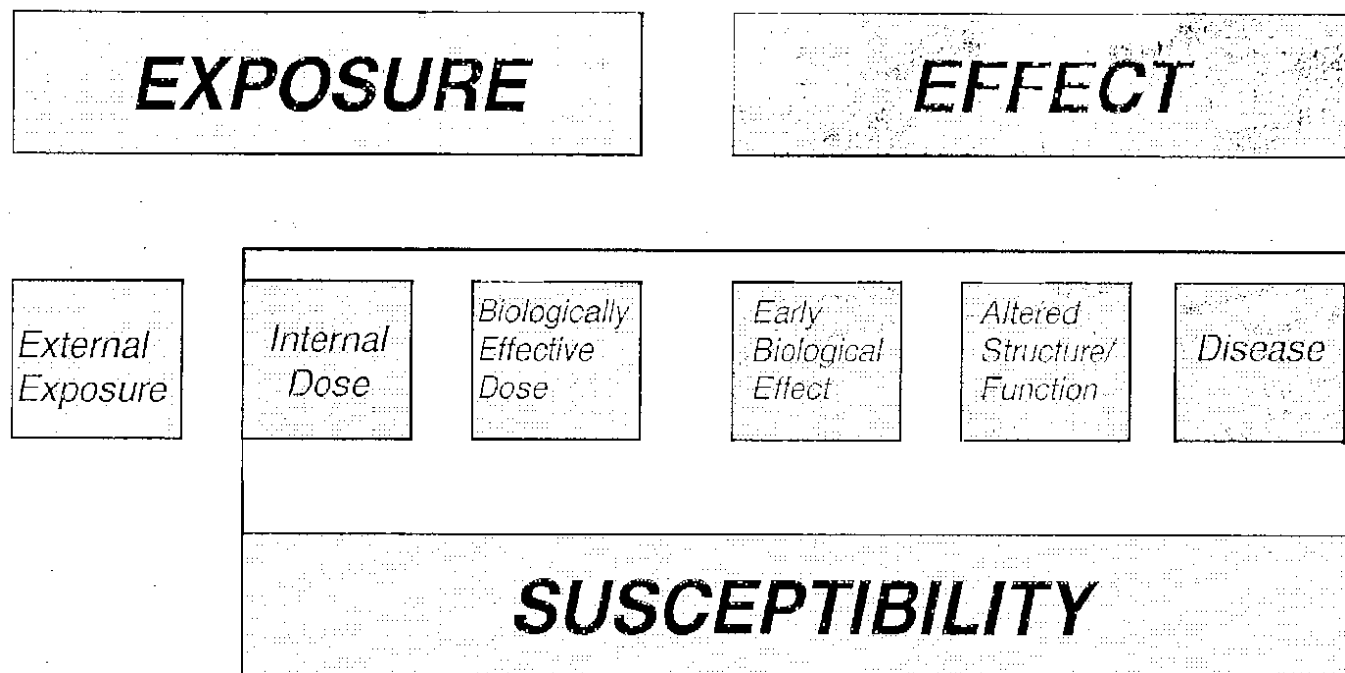
What is Exposure?

- Exposure is the contact over time and space between a person and one or more biological, chemical or physical agents (US NRC, 1991).
- Exposure is concentration of a substance in the human body over time, ($c \times t$).
- Exposure is the area under the concentration-time-curve, (AUC).

From Clearing the Smoke

Exposure or Biomarker Assessment	Definition
External Exposure Marker	A tobacco constituent or product that may reach or is at the portal of entry to the body
Biomarker of Exposure	A tobacco constituent or metabolite that is measured in a biological fluid or tissue that has the potential to interact with a biological macromolecule; sometimes considered a measure of internal dose
Biologically Effective Dose (BED)	The amount that a tobacco constituent or metabolite binds to or alters a macromolecule; estimates of the BED might be performed in surrogate tissues
Biomarker of Potential Harm	A measurement of an effect due to exposure; these include early biological effects, alterations in morphology, structure, or function, and clinical symptoms consistent with harm; also includes "preclinical changes"

Biological Marker Components in Sequential Progression Between Exposure and Disease



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Selection of Biomarkers of Exposure

- Convened panel of PM experts and consultants with significant experience in smoke composition and/or exposure assessment
- Utilized biomarker selection considerations from NRC and N. Benowitz
- Identified candidate smoke constituents
- Chose biomarkers of exposure representative of both smoke phases

Selection Criteria for Biomarkers of Exposure

- “Unique or nearly unique” to tobacco smoke, “so that other sources are minor in comparison”
- Reliable analytical methods available (“easily detectable”)
Benowitz, N.L. Environmental Health Perspectives, 1999, 107(Suppl. 2), 349 – 355
- Representative of either particulate or gas-vapor phase
- Representative of health-relevant constituents
- Sampling to acquire material for analysis only minimally invasive
- Constituent metabolism understood
- Increased in smokers as compared to non-smokers

Biomarkers of Exposure Selected for Use in the Pilot Study

BIOMARKER	SAMPLE MATERIAL	SMOKE CONSTITUENT	SMOKE PHASE (b)
Acetonitrile	Exhalate; urine	Acetonitrile	GVP
Carbon monoxide	Exhalate	Carbon monoxide	GVP
Carboxyhemoglobin	Blood	Carbon monoxide	GVP
Hb adducts of 3- and 4-aminobiphenyl	Blood	3- and 4- Aminobiphenyl	PP
Nicotine and nicotine metabolites (a)	24-hr urine	Nicotine	PP
NNAL and NNAL-glucuronide	24-hr urine	NNK	PP

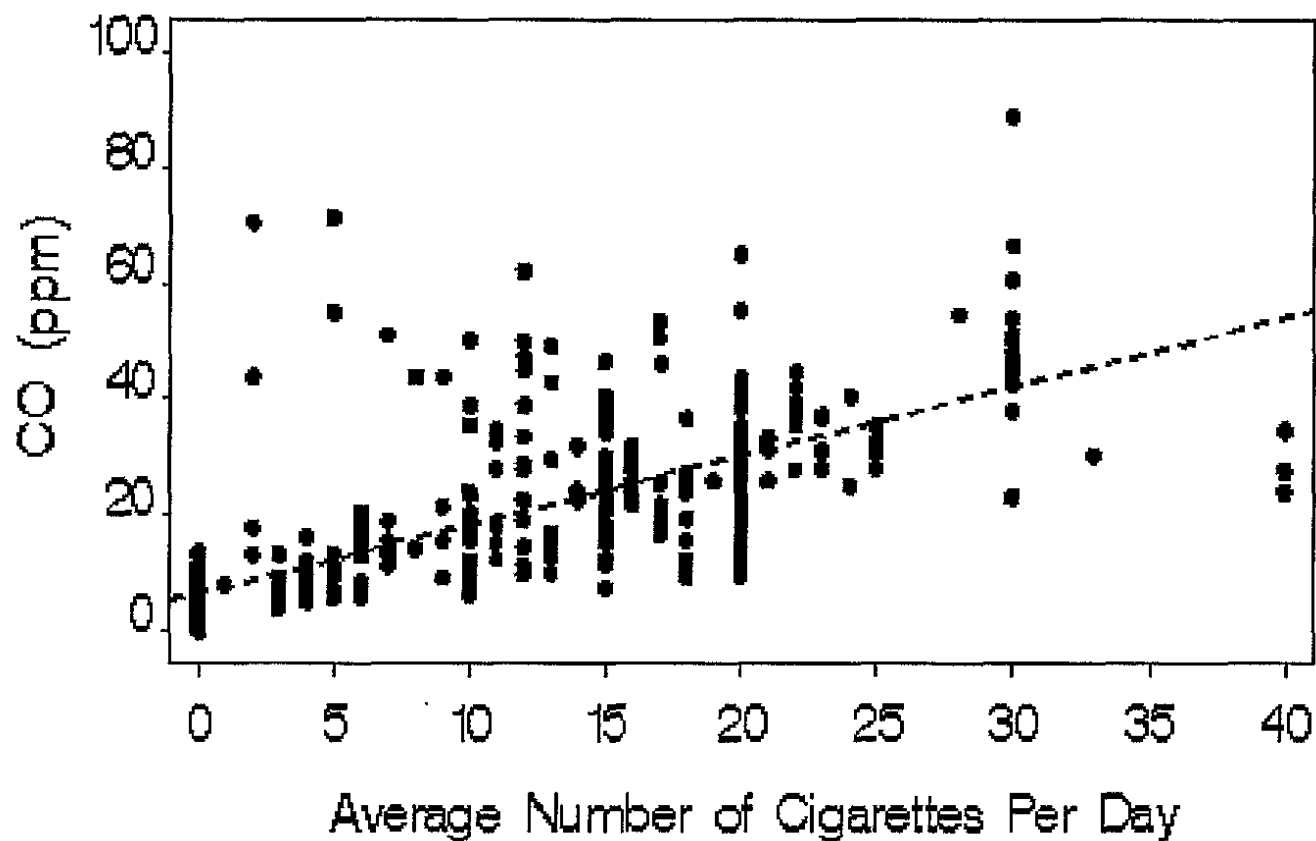
(a)cotinine, 3-hydroxycotinine, nicotine-*N*-glucuronide, cotinine-*N*-glucuronide, and *trans*-3'-hydroxycotinine-*O*-glucuronide,

(b)GVP: gas-vapor phase; PP: particulate phase

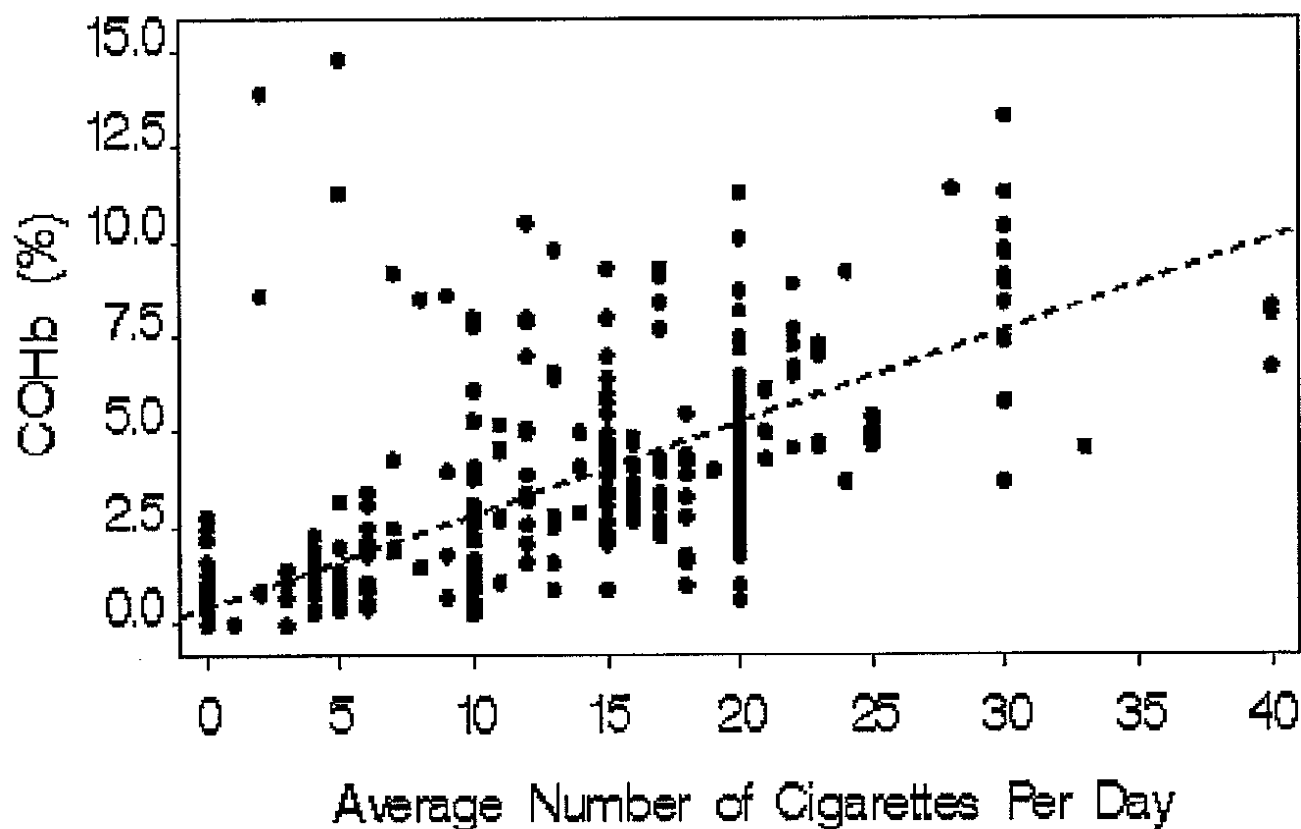
Example of Gas Phase Biomarker: Carbon Monoxide

- Mainstream smoke levels of 1.8 - 13.7 mg/cigt, sidestream 54.1 mg/cigt (1R4F), ETS 0.3 – 33 ppm; but confounders include vehicle exhaust and home heating systems
- Health relevance: diminished oxygen-transport capacity of hemoglobin
- Biological $t_{1/2}$ =
 - * 2 - 3 h for CO in exhalate
 - * ~ 3 h (awake) and ~ 7 h (sleeping) for COHb
- Ratio: smokers to non-smokers:
 - * ~ 8 : 1 for CO in exhalate
 - * ~ 5 : 1 for COHb
- Metabolism: exhaled unchanged; forms adduct with hemoglobin

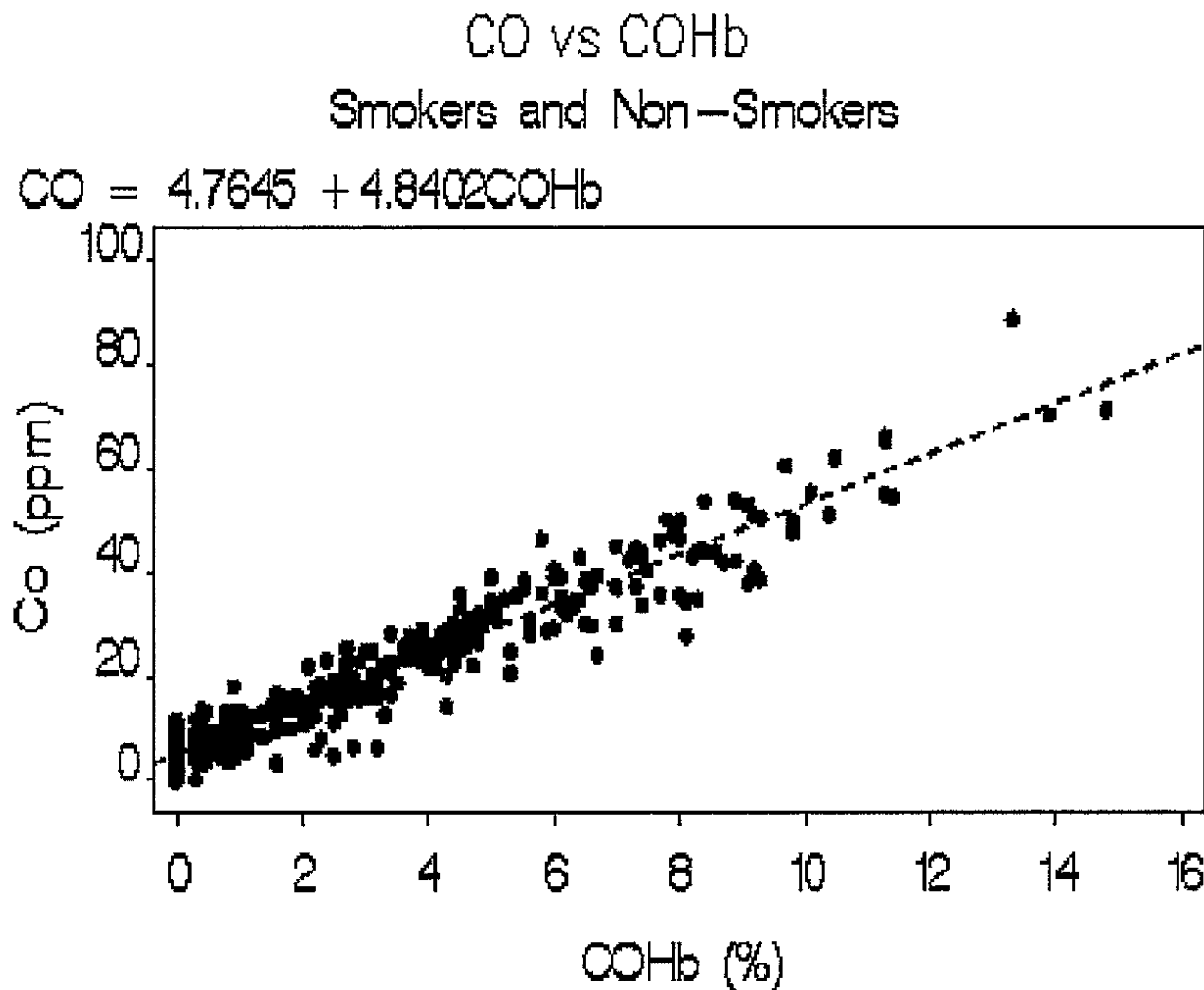
CO (ppm) vs Average Number of CPD
Smokers and Non-Smokers



COHb (%) vs Average Number of CPD
Smokers and Non-Smokers

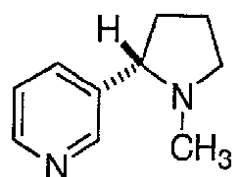


RR. D. Kinser, B. L. Nelson, et al. "Assessment of Human Exposure to Cigarette Smoke Constituents: Pilot Study Results for Carbon Monoxide" Society of Toxicology, March, 2002

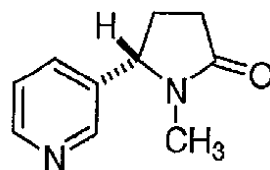


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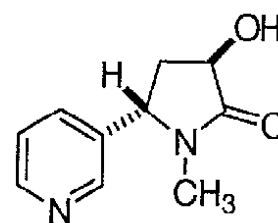
Nicotine Metabolism



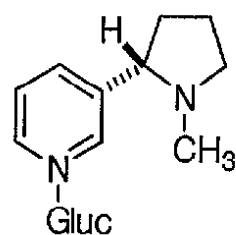
Nicotine



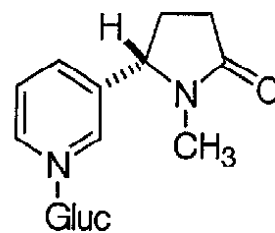
Cotinine



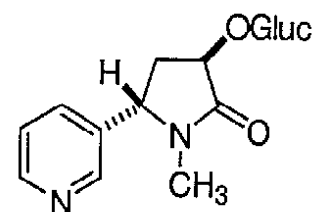
trans-3'-hydroxycotinine



Nicotine-N-Gluc



Cotinine-Gluc



trans-3'-hydroxycotinine-Gluc

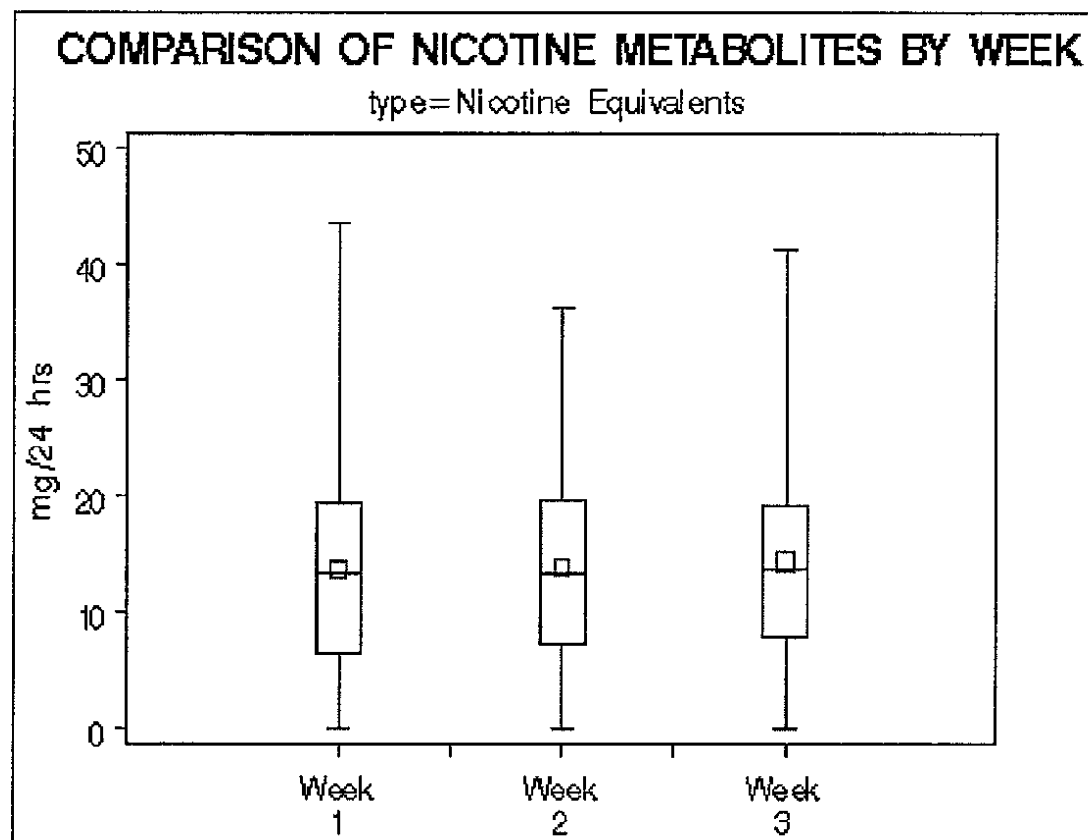
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Nicotine

- Mainstream smoke yields of 0.07 - 1.92 mg/cigt, sidestream: 5.6 mg/cigt (2R1F), ETS: 0 – 90 $\mu\text{g}/\text{m}^3$ (restaurant)
- Biological $t_{1/2}$ (elimination) :
 - * 11 h for nicotine
 - * 19.5 h for cotinine
 - * 6.4 h for 3'-OH-cotinine
 - * 7.2 h for 3'-OH-cotinine-*O*-glucuronide
- Ratio: smokers to non-smokers:
 - * 136 : 1 for nicotine
 - * 302 :1 for cotinine
- Analytical method: LC/MS/MS for nicotine and 5 metabolites



- Box length is interquartile range
- Red square is mean
- Horizontal line is median
- Vertical lines extend to minimum and maximum values

R.D. Kinser, B.L. Nelson, H.-J. Roethig, R.-A. Walk, J. Oey, A.R. Tricker, D.E. Leyden, "Use of Nicotine and NNK Metabolites to Assess Exposure to Cigarette Smoke Constituents: Pilot Study Results" Society for Research on Nicotine and Tobacco, February, 2002.

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Analytical Validation

- PM USA Quality System DRAFT: Conformance to calibration model, accuracy, precision (repeatability and reproducibility), specificity, LOD/LOQ, robustness, stability, system suitability
- Consistent with U.S. Food and Drug Administration Guidance for Industry on Bioanalytical Methods Validation

Biological Validation

- Components: Determination of specificity; exposure-related toxicokinetics and toxico-dynamics; dose-response relationship; biological variation associated with the marker (intra-individual; inter-individuals; group differences); route of exposure; type of health effect; behavioral factors influencing exposure; generation of baseline or normative data
- Pilot study of 69 adult male and female smokers of 3.9 - 6.0 mg tar yield (U.S. Federal Trade Commission methods) and 68 adult male and female non-smokers with repetitive sampling over a 6-week period.

Validation Example: Nicotine, Cotinine, and 3'-OHCotinine

	Nic	Cot	3'-OH-Cot
LLOQ	1.00 ng/mL (6.17 nmol/L)	1.00 ng/mL (5.64 nmol/L)	1.00 ng/mL (5.18 nmol/L)
Inter-Assay Precision (%RSD)			
Low QC	10.8	10.9	11.7
Med QC	5.5	4.6	5.8
High QC	3.3	2.3	2.7
Inter-Assay Accuracy (%DMT)			
Low QC	1.3	3.5	6.5
Med QC	-9.7	-7.6	-7.4
High QC	-11.2	-10.2	-10.2

Table notes: LLOQ = Lower Limit of Quantification; QC = Quality Control Sample
 Low QC = 2 ng/mL; Mid QC = 400 ng/mL; High QC = 750 ng/mL
 %RSD = % relative standard deviation
 %DMT = % deviation of mean from theoretical

Additional Biomarkers of Exposure

- 1,3-Butadiene
 - * 1,3-Butadiene in exhalate
 - * M1 and M2 in urine
- Acrolein
 - * 3-HPMA in urine
- PAHs
 - * Hydroxypyrene and hydroxyphenanthrenes in urine
- Ames test for urine mutagenicity

Currently

- Several biomarkers of exposure have been validated both analytically and biologically
- Additional biomarkers of exposure have been validated analytically
- Analytical methods for additional biomarkers of exposure are in development